Free Radical Biology & Medicine, Vol. 30, No. 10, pp. 1122–1129, 2001

0891-5849/01/$–see front matter

ELSEVIER

PII S0891-5849(01)00519-6

Original Contribution

EFFECTS OF α-TOCOPHEROL ON SUPEROXIDE PRODUCTION AND PLASMA INTERCELLULAR ADHESION MOLECULE-1 AND ANTIBODIES TO OXIDIZED LDL IN CHRONIC SMOKERS

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(*Received* 28 *August* 2000; *Accepted* 23 *February* 2001)

Abstract—Antioxidants have been postulated to exert beneficial effects in atherosclerosis. Atherosclerosis is associated with raised plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1) and autoantibodies against oxidized low-density lipoprotein (oxLDL). It is not known whether antioxidants affect these plasma factors in chronic smokers. In a randomized double-blind placebo-controlled study involving 128 male normolipidemic chronic smokers the effect of a 2-year α-tocopherol treatment (400 IU dL-α-tocopherol daily) on plasma levels of sICAM-1 and autoantibodies against oxLDL was evaluated. In addition, we monitored production of superoxide by leukocytes ex vivo. It was found that compared to nonsmokers (n = 33) plasma levels of IgG but not IgM autoantibodies against oxLDL and concentrations of sICAM-1 in smokers were significantly elevated (30 and 42%, respectively). After supplementation with α-tocopherol concentration of TBARS in plasma and in vitro oxidizability of LDL had decreased, but autoantibodies and sICAM-1 had not changed. Production of superoxide was not different between α-tocopherol- and placebo-treated smokers. It is concluded that in chronic smokers, long-term treatment with α-tocopherol does not normalize the raised levels of sICAM-1 and autoantibodies against oxLDL, both risk factors for initiation or progression of cardiovascular disease, despite a decrease in in vitro oxidizability of LDL. © 2001 Elsevier Science Inc.

Keywords—Smoking, Antioxidants, Atherosclerosis, Oxidized lipoproteins, Cell adhesion molecules, Superoxide, Autoantibodies, α-tocopherol, Free radicals

INTRODUCTION

Smoking is a major risk factor for the development of atherosclerosis [1] but the underlying mechanisms are not clear. Smoking has been associated with increased oxidative stress [2,3], abnormal endothelial function [4–6], and increased leukocyte adhesion [7,8]. The latter is a critical step in the initiation of atherosclerosis [9] and is mediated by adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Compared to nonsmokers, smokers have significantly higher plasma concentrations of soluble ICAM-1 (sICAM-1) [7,10,11] and autoantibodies against oxidized low-density lipoprotein (oxLDL) [12], although the latter may be true only in smokers with cardiovascular disease (CVD) [13] or hypercholesterolemia [4,5]. Plasma concentration of sICAM-1 and level of autoantibodies against proatherogenic oxLDL are suggested to be useful markers of asymptomatic [12,14,15] and symptomatic atherosclerosis [13,16–19] and predictive of CVD [20–22].

Antioxidants have been postulated to exert beneficial effects in atherosclerosis, though this hypothesis is still a matter of debate [23–25]. Supplementation with the lipid-soluble antioxidant vitamin α-tocopherol reportedly has been shown to increase the resistance of LDL against oxidative modification in vitro [26], inhibit monocyte-endothelial adhesion [27–30], preserve endothelium-dependent vaso-relaxation [31], and inhibit leukocyte su-

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peroxide and cytokine production [32–34]. Martin et al. [35] demonstrated an inhibitory effect of α-tocopherol on induction of sICAM-1 in human aortic endothelial cell culture, but it is not known whether α-tocopherol affects plasma concentration of sICAM-1 in subjects at risk for CVD. Very recently, Heitzer et al. [4] demonstrated suppression of autoantibody titers against oxLDL following supplementation of α-tocopherol in hypercholesterolemic smokers.

We conducted a 2 year randomized double-blind placebo-controlled study in normolipidemic male chronic smokers to evaluate the effect of α-tocopherol treatment (400 IU dL-α-tocopherol per day) on plasma levels of sICAM-1 and autoantibodies against oxLDL. In addition, we monitored in vitro oxidation of LDL and measured superoxide production by polymorphonuclear cells and in diluted whole blood after the intervention.

MATERIALS AND METHODS

Subjects

Subjects were male cigarette smokers (59.9 ± 6.0 years, $n = 128$) who had smoked an average of 42 ± 7 years, and male nonsmokers (49.2 ± 11.3 years, $n = 33$, $p < .05$ versus smokers), all recruited from the general population. None of the subjects suffered from diabetes mellitus or current illness interfering with participation. Subjects did not use antilipidemic drugs, vitamin K antagonists, or (multi)vitamin, vitamin E, vitamin C, β-carotene, garlic, or fish oil supplements. The study was designed as a 2 year randomized double-blind placebo-controlled trial. Smokers received either capsules containing a daily dose of 400 IU (268 mg) vitamin E as dL-α-tocopherol ($n = 64$) or placebo capsules ($n = 64$), provided by Hoffman La Roche Ltd (Basel, Switserland). Pack-years of smoking (calculated by multiplying duration of smoking by the number of cigarettes smoked per day divided by 20) did not differ between α-tocopherol and placebo group (36 = 21 and 38 = 20, respectively). Fasting blood samples for analysis of different parameters were collected before and after the intervention. Written informed consent was obtained from all participants, and the protocol of the study was approved by the ethics committees of University Medical Center Nijmegen and Wageningen University.

sICAM-1

Concentration of sICAM-1 in EDTA plasma was measured with a sandwich ELISA described elsewhere [36]. Monoclonal antibody HM2 and biotin-labeled monoclonal antibody HM1 (both gifts of Dr. W. Buurman, University of Maastricht, The Netherlands) were used as capture and detection antibody, respectively. The lower detection limit of the assay was 400 pg/ml. To minimize analytical variations, all determinations of sICAM-1 in this study were performed on one day, using the same batch of reagents.

Antibodies to LDL

IgG and IgM antibodies against oxLDL in EDTA plasma were assayed by ELISA, essentially as described by others [37]. In detail, sterile native LDL was isolated from 20 ml of EDTA blood by single spin density gradient ultracentrifugation [38], and dialyzed against 10 mM phosphate buffer pH 7.4 containing 0.9% NaCl. For oxidation, LDL was incubated with 20 μM $CuSO_4$ for 15 h at 37°C in a shaking water bath. Half of a microtitre plate (Nunc-ImmunoPlate Maxisorp, Roskilde, Denmark) was coated with native LDL and the other half with oxLDL, both at 7.5 μg/ml antigen protein in PBS containing butyl-hydroxytoluene (BHT; 20 μM) and EDTA (0.27 mM) to prevent oxidation of LDL in the ELISA plate, and incubated overnight at 4°C. Plates were washed four times with PBS containing 0.05% (w/v) Tween 20 (Sigma-Aldrich, Steinheim, Germany) (PBS-Tween), and the remaining binding sites were blocked by incubating for 2 h at room temperature with 1% (w/v) human serum albumin (HSA; Behringwerke AG, Marburg, Germany) in PBS containing 20 μM BHT and 0.27 mM EDTA. After washing, 100 μl of plasma diluted with PBS containing 0.1% HSA (1:10 and 1:100 for IgM and IgG, respectively) was added. Plates were incubated for 2 h at room temperature and washed four times with PBS-Tween. For detection of autoantibodies, peroxidase-conjugated antibody (1:5000) from goat specific for human IgG or IgM (Sigma-Aldrich) was applied for 1 h at room temperature. After extensive washing with PBS-Tween, peroxidase activity was developed using tetramethylbenzidine as a substrate and terminated by addition of 2 M H_2SO_4. The optical density was measured at 450 nm (OD_{450}) with a multilabel counter (Victor^2 1420, Wallac, Turku, Finland). The results were expressed as the mean OD values from duplicate measurements, and the antibody titer against oxLDL was calculated by subtracting the binding to native LDL from the binding to copper-oxidized LDL. With this subtraction method our assay not only corrects for specific binding to native LDL but also for nonspecific binding of each sample to the ELISA wells. To minimize analytical variations, all determinations of autoantibodies against oxLDL in this study were performed on one day, using the same batches of native and oxLDL, and the same batch of reagents. To prove the accuracy of the assay, we performed inhibition experiments using native LDL or oxLDL as inhibitors. Fourteen plasma samples were

incubated at room temperature for 2 h with either 35 μg/ml native LDL, or 35 μg/ml oxLDL, or without inhibitor. These samples were then analyzed for antibodies against oxLDL as described above. It was found that preincubation with native LDL did not affect the detection of antibodies, but preincubation with oxLDL inhibited the detection of IgG as well as IgM antibodies against oxLDL (67.5 ± 16.3% inhibition, $p < .0001$ and 62.1 ± 24.2% inhibition, $p < .0001$, respectively).

Superoxide production

Production of superoxide by isolated polymorphonuclear cells (PMN) in response to phorbol 12-myristate 13-acetate (PMA; 50 ng/ml; Sigma-Aldrich) was assessed as reduction of ferricytochrome *c* [39] and by monitoring luminol-enhanced peroxidase-catalyzed chemiluminescence (CL) [34]. PMN were isolated from heparinized blood as described earlier [34], washed twice in PBS, and suspended in Hanks Balanced Salt Solution (HBSS, Life Technologies, Paisley, UK) supplemented with 0.25% HSA. PMN suspensions had a purity of > 99%. Cell concentration was assessed using a cell counter (Coulter Electronics, Luton, UK). Maximum rate of reduction of cytochrome *c* was determined at 37°C at 550 nm on a thermostatted Perkin-Elmer Lambda 12 spectrometer (Perkin-Elmer, Norwalk, CT, USA) and expressed in nmol/min per 10^6 PMN, using 21.1 mmol/l/cm as the extinction coefficient for (reduced-oxidized) cytochrome *c* [40]. Due to technical reasons, reduction of cytochrome *c* was not performed for all subjects. Peak CL-activity of PMN was measured on a Victor 1420 multilabel counter (Wallac, Turku, Finland). Each well contained 2×10^5 cells, 50 μM luminol (Sigma-Aldrich), and 4.5 U/ml horseradish peroxidase (hrp; Sigma-Aldrich). Integrated CL-activity of heparinized whole blood (final dilution 3000 × in HBSS/HSA 0.25%) during 20 min following PMA stimulation (50 ng/ml) was measured identically in a microplate luminometer MicroLumat Plus LB96V (Berthold Co, Wildbad, Germany) as described earlier [34]. Hrp was included in the CL assay in order to overcome peroxidase deficiency extracellularly [41]. PMA-induced reduction of cytochrome C by PMN and PMA-induced chemiluminescence activity of PMN and of whole blood could be totally quenched with superoxide dismutase (50 U/ml, final concentration) and prevented with the protein kinase C inhibitor staurosporine (50 nM, final concentration), indicating the exclusive reflection of superoxide production and the specificity of these assays, respectively (data not shown).

Because measurement of cellular activities over a 2-year interval is difficult to standardize, production of superoxide was measured after the intervention only. To exclude analytical variations, equal numbers of placebo- and α-tocopherol-treated subjects were assayed at the same time.

Other methods

Cholesterol and triglyceride concentrations in serum were determined by enzymatic methods (Boehringer-Mannheim, Mannheim, Germany) on a Hitachi 747 analyzer (Hitachi, Tokyo, Japan). Serum high-density lipoprotein (HDL) cholesterol was determined after precipitation of LDL, very low-density lipoprotein and chylomicrons using phosphotungstate/Mg^{2+} [42]. LDL-cholesterol in serum was calculated using the Friedewald formula [43]. Concentrations of α-tocopherol were analyzed by reversed-phase high-performance liquid chromatography [44]. Susceptibility of LDL, isolated from EDTA plasma supplemented with saccharose before storage at −80°C, to in vitro oxidation, was monitored by the change in absorbance at 234 nm as described by Esterbauer et al. [45] and as modified by Princen et al. [46]. Lag time, defined as the time interval between the intercept of the linear least-square slope of the absorbance curve with the initial-absorbance axis, was taken as a measure of resistance to oxidation. Thiobarbituric acid reactive substances (TBARS) in plasma were determined using a method described by Conti et al. [47], modified by replacing the recording of the fluorescence spectrum of the thiobarbituric acid-malondialdehyde complex by a simple fluorimetric measurement (measurement on a Shimadzu RFF-500 fluorimeter; excitation: 530 nm; emission 545 nm; bandwith 5 for both). By occasionally recording the fluorescence between 500 and 600 nm and measuring only the peak at 533 nm keeping a constant interval of 14 nm between excitation and emission wavelengths, we found that compounds unspecifically reacting with the diethylthiobarbituric acid were absent in the samples tested. In this way specificity comparable to an assay with HPLC prepurification can be expected.

Data analysis and statistics

The computer program ASTUTE (Microsoft Inc., Redmond, WA, USA) was used for the analysis. Data are presented as means ± SD and evaluated by using Student's *t*-test for paired and unpaired data. Linear regression analysis and Pearson correlation test were performed to examine the relationship between variables.

RESULTS

Lipid and lipoprotein profiles, LDL oxidation and α-tocopherol concentrations

Data summarized in Table 1 show that in smokers plasma concentrations of total cholesterol, triglycerides,

Table 1. Lipid and Lipoprotein Profiles, α-Tocopherol Concentrations and Lagtime of LDL Oxidation of Study Groups

		Smokers before intervention		Smokers after intervention[b]	
	Nonsmokers[a] (n = 33)	Placebo (n = 64)	α-Tocopherol (n = 64)	Placebo (n = 64)	α-Tocopherol (n = 64)
Plasma					
Total cholesterol (mmol/l)	5.48 ± 1.19[c]	6.13 ± 1.01	5.89 ± 0.94	5.85 ± 0.79[d]	5.98 ± 0.95
Triglycerides (mmol/l)	1.20 ± 0.66[d]	1.65 ± 0.62	1.72 ± 0.96	1.58 ± 0.60	1.78 ± 0.91
HDL cholesterol (mmol/l)	1.35 ± 0.30[c]	1.18 ± 0.31	1.21 ± 0.43	1.20 ± 0.30	1.15 ± 0.33[e]
LDL cholesterol (mmol/l)	3.70 ± 0.98[c]	4.24 ± 0.95	3.96 ± 0.88	3.99 ± 0.82[d]	4.08 ± 0.86
α-tocopherol (mg/l)		13.2 ± 2.9	13.0 ± 2.8	14.2 ± 3.2[d]	31.2 ± 10.4[e]
TBARS (μmol/l)		0.56 ± 0.12	0.65 ± 0.14	0.49 ± 0.12	0.55 ± 0.13[e]
LDL					
α-tocopherol (mg/g LDL protein)		5.63 ± 1.61	5.48 ± 1.33	5.81 ± 1.86	10.65 ± 3.37[e]
Lagtime of LDL oxidation (min)		87.9 ± 8.6	89.4 ± 11.8	86.0 ± 9.8[d]	105.1 ± 13.7[e]

[a] t-test versus smokers before intervention (n = 128); [b] paired t-test vs. initial values; [c] $p < .05$; [d] $p < .01$; [e] $p < 0.001$; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

and LDL cholesterol were significantly higher and plasma concentration of HDL cholesterol significantly lower compared to nonsmokers. Among smokers, the placebo and α-tocopherol group did not differ in baseline plasma lipids, lipoproteins, and TBARS, α-tocopherol concentrations in plasma and LDL, and resistance of LDL to in vitro oxidation. Supplementation with α-tocopherol resulted in a large increase in α-tocopherol concentrations in plasma and in LDL (+140%, $p <$ .0001 and +94%, $p < .0001$, respectively). In the placebo group the concentration of α-tocopherol in plasma increased slightly (+7%, $p < .01$), whereas no change in LDL was observed. During the intervention period plasma lipids and lipoproteins were stable except for a small but significant change in total cholesterol (−5%, $p < .01$) and LDL cholesterol (−6%, $p < .01$) in the placebo group and HDL cholesterol (−5%, $p < .05$) in the α-tocopherol group. Plasma concentration of TBARS did not change in the placebo group but decreased significantly in the α-tocopherol group (−15%, $p < .05$). As expected, lagtime of LDL oxidation increased markedly following supplementation with α-tocopherol (+18%, $p < .0001$). In the placebo-treated group lagtime of LDL oxidation decreased slightly but significantly (2%, $p < .01$).

sICAM-1 and antibodies against oxidized LDL

Table 2 shows that in smokers plasma levels of sICAM-1 and titers of IgG antibodies against oxLDL were higher than in nonsmokers (+42%, $p < .001$ and +30%, $p < .01$, respectively). IgM antibodies were not different between smokers and nonsmokers. There were no significant associations of sICAM-1 concentrations or antibody titers with age, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, or with lagtime of LDL oxidation in vitro (data not shown). Among smokers, the placebo and α-tocopherol group did not differ in baseline values of sICAM-1 and antibodies against oxLDL (Table 2). After the intervention period, no change was observed in the plasma concentrations of sICAM-1 or IgG antibodies against oxLDL. IgM antibodies increased slightly in both groups, but the increase was significant in the placebo group only.

Superoxide production

Table 3 shows production of superoxide by PMN of smokers in response to PMA, measured as peak CL-activity and as maximum rate of reduction of cytochrome c. Both parameters did not differ between both groups

Table 2. Plasma Levels of sICAM-1 and IgG and IgM Autoantibodies Against Oxidized LDL of Study Groups

		Smokers before intervention		Smokers after intervention	
	Nonsmokers (n = 33)	Placebo (n = 64)	α-Tocopherol (n = 64)	Placebo (n = 64)	α-Tocopherol (n = 64)
sICAM-1 (ng/ml)	112.3 ± 25.8[c]	156.4 ± 52.1	163.2 ± 42.1	156.5 ± 56.7	162.2 ± 45.3
IgG antibodies (OD_{450})	0.201 ± 0.084[b]	0.256 ± 0.169	0.272 ± 0.146	0.256 ± 0.150	0.282 ± 0.166
IgM antibodies (OD_{450})	0.500 ± 0.289	0.455 ± 0.295	0.510 ± 0.338	0.481 ± 0.302[a]	0.547 ± 0.368

[a] $p < .05$ (paired t-test versus initial values); [b] $p < .01$; [c] $p < .001$ (t-test vs. smokers (n = 128) before intervention); sICAM-1 = soluble intercellular adhesion molecule-1; IgG = immunoglobulin G; IgM = immunoglobulin M; LDL = low density lipoprotein; OD_{450} = optical density at 450 nm.

Table 3. Production of Superoxide by PMN and in Whole Blood of Smokers Treated with Placebo or α-Tocopherol

	Placebo-treated	α-Tocopherol-treated
PMN		
Cytochrome *c* reduction (nmol/min)	0.159 ± 0.035 (n = 55)	0.161 ± 0.036 (n = 53)
Chemiluminescence ($\times 10^{-3}$ RLU/s)	201.5 ± 46.9 (n = 64)	197.0 ± 42.9 (n = 64)
Whole blood		
Chemiluminescence ($\times 10^{-6}$ RLU)	1.51 ± 0.54 (n = 64)	1.59 ± 0.61 (n = 64)

PMN = polymorphonuclear cells; RLU = relative light units.

after the intervention. Also, integrated CL-activity of whole blood of smokers did not differ between α-tocopherol and placebo group. CL-activity of PMN significantly correlated with reduction of cytochrome *c* ($r = 0.49$, $p < .0001$, $n = 108$). CL of whole blood was weakly associated with reduction of cytochrome *c* by PMN ($r = 0.28$, $p = .003$, $n = 108$).

DISCUSSION

In the present study, we show that plasma levels of IgG autoantibodies against oxLDL and sICAM-1 in elderly male chronic smokers are elevated compared to nonsmokers. Treatment with α-tocopherol for 2 years at a daily dose of 400 IU significantly enhanced resistance of LDL to in vitro oxidation and decreased plasma concentration of TBARS. However, no change in plasma levels of IgG autoantibodies against oxLDL and sICAM-1 was observed. This is an important observation because it implies that elderly normolipidemic chronic smokers may have increased exposure to oxLDL, as reflected by raised levels of autoantibodies, but do not benefit from α-tocopherol treatment. To our knowledge, these are the first conclusive data on plasma levels of sICAM-1 and autoantibodies against oxLDL obtained from a well-designed (randomized double-blind placebo-controlled) long-term trial of α-tocopherol in chronic smokers.

Previously, others observed normal plasma levels of IgG autoantibodies against oxLDL in normolipidemic smokers but their study did not have enough power to detect significant differences in autoantibody levels between smokers and controls [4]. The same group reported that in hypercholesterolemic smokers levels of IgG autoantibodies against oxLDL were elevated compared to normolipidemic nonsmokers and decreased after treatment with α-tocopherol [4,5]. In the present study, including large numbers of subjects, we established that levels of IgG autoantibodies against oxLDL in elderly chronic smokers are elevated compared to nonsmokers, and we observed no effect of α-tocopherol treatment. In our population smokers were significantly older than nonsmokers but lipid and lipoprotein levels were in the normal range [48], and, in agreement with other studies [13,49–51], levels of autoantibodies were not related to age, plasma lipids, and lipoproteins. It seems therefore that, depending on the severity of hypercholesterolemia, α-tocopherol may reduce the level of autoantibodies against oxLDL in chronic smoking, but not to such a degree that normal levels are reached.

Though clinical studies suggest an association with CVD [12,13,19,22], the biological significance of autoantibodies against oxLDL is not well established. Recently, an inverse relationship between circulating oxLDL and autoantibody titers against oxLDL in healthy subjects was demonstrated [52], suggesting that autoantibodies may play a role in maintaining the low level of plasma oxLDL. The raised levels of circulating autoantibodies against oxLDL in chronic smokers may then reflect increased exposure to oxLDL due to oxidative stress [2]. Increased circulating products of lipid peroxidation have been demonstrated in long-term smokers [3,53,54], and smoking cessation increases the resistance of LDL to in vitro oxidation [55]. On the other hand, in our study we did not find a correlation between LDL oxidizability and autoantibody titers, and, following α-tocopherol supplementation LDL oxidizability and plasma concentration of TBARS decreased in accordance with literature [26,33,56], but antibody titers were not affected. This may indicate that the antibodies are generated in response to antigenic stimuli not affected by α-tocopherol supplementation, such as LDL material already deposited in the wall of arteries. Furthermore, oxidizability of LDL is determined in vitro in an assay in which most factors relevant for oxidation of LDL in vivo are lacking and the assay may not be a good indicator of the atherogenic risk [57]. However, the apparent inconsistency may also be related to circulating oxLDL-containing immune complexes, which in diabetes mellitus have been shown to interfere with the assay of free oxLDL autoantibodies [58]. Whether this phenomenon plays a role in long-term smoking is not yet clear. In the present study free antibody concentrations are assayed. A technique that determines both quality and quantity of Ig in oxLDL-containing immune complexes, and thus en-

ables correction of free antibody levels for the presence of immune complexes, is not yet available.

Cigarette smoking has been associated with increased expression of ICAM-1 on the endothelium [8,59] and increased endothelial cell adhesion [8]. In agreement with other studies we observed elevated levels of sICAM-1 in smokers (+42%) compared to nonsmokers [7,10,11] and no relation of sICAM-1 with age or plasma lipids and lipoproteins [16,60]. Because smoking is known to impair endothelial release of nitric oxide (NO) [61] and NO is important in the control of adhesion molecule expression by inhibiting mobilization of nuclear transcription factor κB (NFκB) [62], the elevated levels of sICAM-1 in smokers might be related to an impaired endothelial release of NO. α-Tocopherol might influence endothelial expression of adhesion molecules via two mechanisms: via inhibition of mobilization of NFκB [63], and/or via scavenging oxygen radicals, thereby preventing NO catabolism [64,65]. It was previously reported that α-tocopherol reduced the induction of sICAM-1 in human aortic endothelial cells exposed to native LDL in vitro [35]. To the best of our knowledge, this is the first study addressing the effect of α-tocopherol on sICAM-1 in vivo. We found no effect of α-tocopherol on sICAM-1 levels in smokers following supplementation of α-tocopherol. Though we have previously shown suppression of PMA-induced protein kinase C-dependent production of superoxide by PMN after short-term treatment of nonsmoking human subjects with α-tocopherol [34], in the present study we found no difference in ex vivo production of superoxide by PMN of smokers treated with placebo or α-tocopherol. Cellular α-tocopherol content very likely is increased in the supplemented group, because both plasma and LDL α-tocopherol concentration were increased. Possibly, during the 2 year supplementation period, cellular physiology has adapted to the elevated α-tocopherol content. On the other hand, α-tocopherol may not be efficient enough in conditions of oxidative stress, such as in smoking.

We conclude that, even at normal plasma lipids and lipoproteins, elderly male chronic smokers have increased plasma levels of sICAM-1 and IgG autoantibodies against oxLDL, which are risk factors for initiation or progression of cardiovascular disease. Treatment with α-tocopherol for 2 years does not normalize the raised levels of these risk factors, despite a decrease in in vitro oxidizability of LDL.

Acknowledgements — The authors gratefully acknowledge the expert technical assistance of Mrs. H. Toenhake and Mrs. M. Hectors.

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ABBREVIATIONS

CL—chemiluminescence
CVD—cardiovascular disease
EDTA—ethylenediaminetetra-acetate
ELISA—enzyme-linked immuno sorbent assay
HBSS—Hanks' balanced salt solution
HDL—high-density lipoprotein
hrp—horseradish peroxidase
HSA—human serum albumin
ICAM-1—intercellular adhesion molecule-1
IgG—immunoglobulin G
IgM—immunoglobulin M
IU—international units
LDL—low density lipoprotein
NFκB—nuclear transcription factor κB
NO—nitric oxide
OD_{450}—optical density at 450 nm
oxLDL—oxidized low density lipoprotein
PBS—phosphate buffered saline
PMA—phorbol 12-myristate 13-acetate
PMN—polymorphonuclear cells
RLU—relative light units
sICAM-1—soluble intercellular adhesion molecule-1
VCAM-1—vascular cell adhesion molecule-1